Dystrophin Mutations Associated with Duchenne and Becker Muscular Dystrophy with Thermodynamic Analysis Using Differential Scanning Calorimetry

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*A statistical study on dystrophins recorded amino acid mutations that lead to Dystrophin's non-function associated with muscular dystrophy. I hypothesize that certain key residues have a higher frequency for a mutation occurring than others in dystrophin. I also hypothesize that through this statistical analysis the reasoning of why certain mutations cause Duchenne muscular dystrophy (DMD: severe) vs Becker muscular dystrophy (BMD: not so severe) can be determined. The analysis was completed through correlating the frequency of certain types of mutations and relating them to the location, the diagnosis, and the overall functionality of the protein. Secondly, I hypothesize that through thermodynamic processes on certain mutant construction of dystrophin, correlation of structure and function can be tested directly.**

**Data from the thermodynamic constructs are still being gathered and analysis. Predicted date of thermodynamic construct data publication: Dec 2015.*

1. Introduction

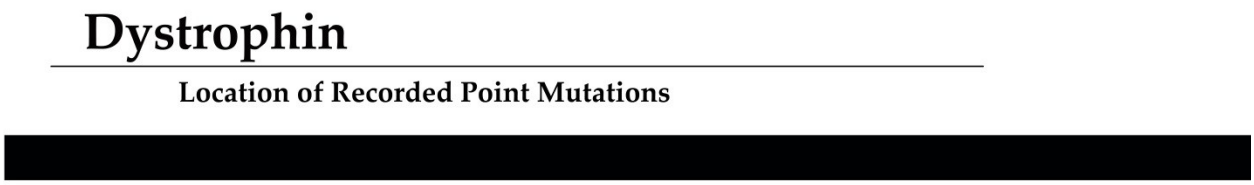
Muscular dystrophy is a class of inherited diseases that causes the muscle tissue to weaken or lose the ability to function. The two diseases, Duchenne and Becker muscular dystrophy originate from the same gene but have drastically different severities. Duchenne patients usually die wheelchair bound in their early 20s while Becker patients live long lives with minor symptoms. Understanding genetically why these two diseases vary will be beneficial for researchers to develop a treatment or cure to the crippling Duchenne's disease. Dystrophin is a

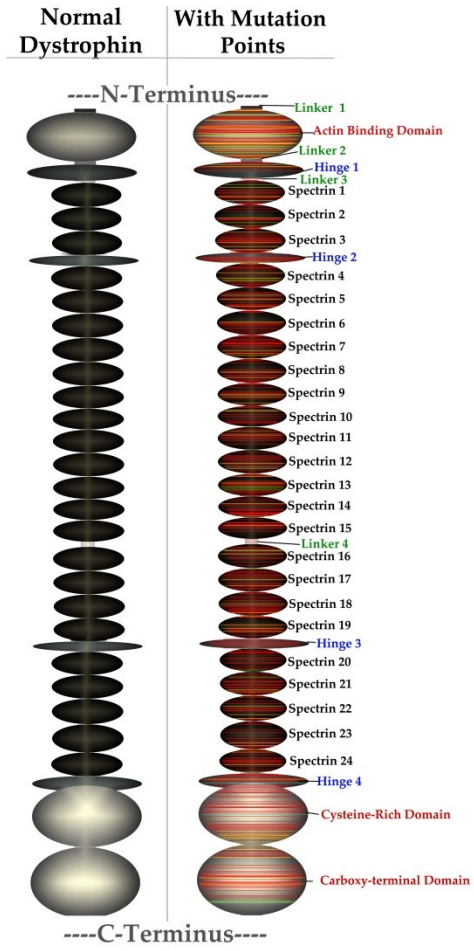
long protein that acts as a shock absorber between muscle cells. Dystrophin links actin and dystroglycans of the sarcolemma and is key to preventing damage in the cells (Ishpekova, 1999). In Duchenne patients the dystrophin fails to function or fails to be produced. The lack of dystrophin or its function is catastrophic to the muscle tissue. Damage is easy created to muscle for the cells lack the natural "shock absorber". In recent studies Le Rumeur and his team (2010) found dystrophin also assists in regulating calcium levels in muscle cells. The regulation of calcium in muscle cells is key to their function (Le Rumeur, 2010). Without particular calcium concentrations muscle cells fail to repair themselves. The constant damage to cells also causes scar tissue to develop in the connective tissue (Bhattacharya, 2014). The lack of repair and buildup of scar tissue causes the loss of function in muscle tissue. Duchenne muscular dystrophin is only seen in young boys. This is because the dystrophin gene (DMD) is an X-linked protein.

2. Methods

Using Leiden Muscular Dystrophy Mutation Database all the mutations (substitution, deletion, insertion, frame shift, and duplication) pertaining to DMD and BMD were mapped out in the structure of dystrophin; the control being the normal strand sequence for dystrophin. Each mutation's original source was validated to assure the mutations from the database were authentic. Leiden Muscular Dystrophy Mutation Database pools documented mutations from around the world. Validations of mutations are as follows: originated from human being, a patient diagnosed with muscular dystrophy, from a licensed and creditable institution. Once mutations were validated, they were then mapped on the structure of dystrophin pertaining to the protein's domains. Frequency (number of recorded incidents for that specific mutation) on each amino acid was then documented and statically analyzed using random and probability factors.

3. Results





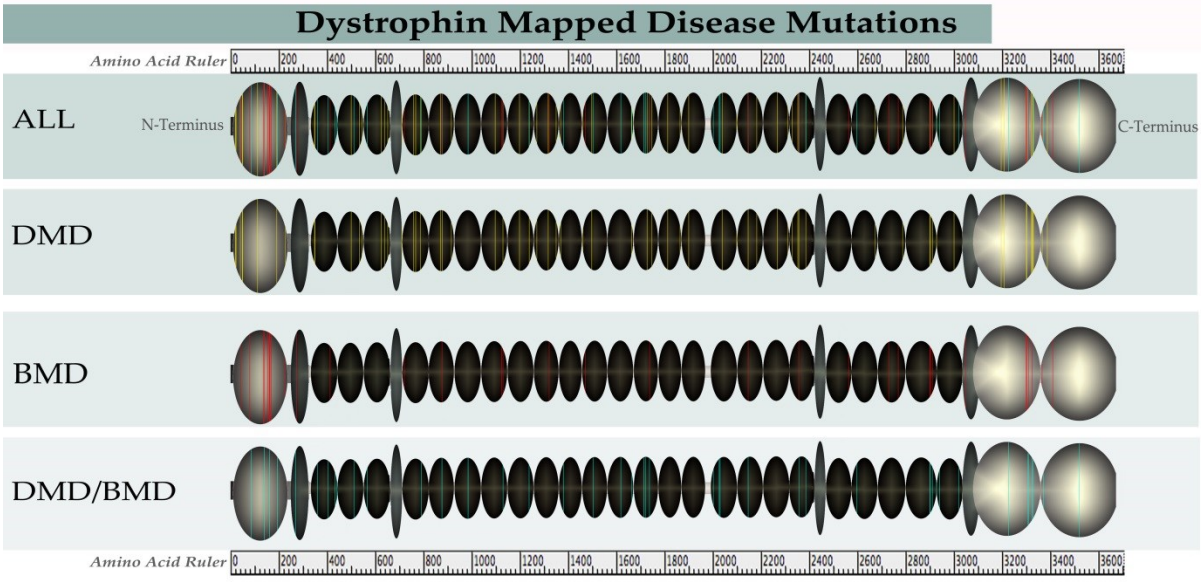
Color Key / Regions	Frame-shifts	Substitutions	Insertion and deletions	Mutation total/Amino acid total	Percentage %
Linker 1	1	-	-	1/13	7.69
Actin Binding Domain	50	24	1	75/226	33.19
Linker 2	-	-	-	0/14	0
Hinge 1	6	2	-	8/110	7.27
Linker 3	1	-	-	1/10	10
Spectrin 1	16	6		32/110	29.10
Spectrin 2	15	3	1	19/108	17.59
Spectrin 3	17	4	-	21/110	19.10
Hinge 2	7	1	-	8/49	16.33
Spectrin 4	15	6	-	21/110	19.10
Spectrin 5	15	3	-	18/105	17.14
Spectrin 6	13	1	-	14/110	12.73
Spectrin 7	18	3	-	21/108	19.44
Spectrin 8	15	2	-	17/108	15.74
Spectrin 9	12	3	-	15/103	14.56
Spectrin 10	10	2	-	12/95	12.63
Spectrin 11	9	3	-	11/104	10.58
Spectrin 12	17	2	-	19/107	17.76
Spectrin 15	15	4	-	19/101	18.81
Spectrin 14	21	2	-	23/95	13.68
Spectrin 15	12	1	-	13/98	13.27
Linker 4	2	-	-	2/17	11.76
Spectrin 16	10	3	-	13/109	11.93
Spectrin 17	17	2	-	19/106	17.93
Spectrin 18	16	2	-	18/109	16.51
Spectrin 19	13	5	-	18/104	17.31
Hinge 3	7	-	-	7/46	15.22
Spectrin 20	12	1	-	13/106	12.26
Spectrin 21	17	3	-	20/108	18.52
Spectrin 22	13	3	-	16/115	13.91
Spectrin 23	13	3		16/128	12.5
Spectrin 24	15	5	-	20/108	18.52
Hinge 4	15	1	1	17/71	23.94
Cysteine Domain	40	10	1	51/268	19.03
Carboxy-Terminal	29	8	1	38/324	11.72

Figure 1 and Table 1. Displayed are the validated recorded mutations for both DMD and BMD on the structure of dystrophin. The width of the colored lines is a pixel. Every pixel represents an amino acid. The graphic composed is a precise size display of each domain of dystrophin in regards to the number of amino acids it contains. The domains are labeled accordingly. The colored lines for the mutations correspond to the type of mutation; red (frame-shift), yellow (substitutions), blue (insertion or deletion). The table is the number of mutation recorded respectfully to each region.

Figure 2 and Table 2. Above displays only the substitution mutations for DMD and BMD on dystrophin. The size of the graphic is still a precise representation of domain size. There is an amino acid ruler placed above and below the graphics to be used as guides to finding desired

Substitution Mutations with Repect to DMD and BMD

Dystrophin Mapped Disease Mutations



Disease Mutation Locations and Number of Recorded Occurences

DMD	Amino Acid	24	26	27	44	53	54	116	196	232	355	353	505	539	545	630	645	658	715	764	773?	793?	868
	Recorded #	2											5					5					
	Amino Acid	882	921	1109	1136	1245	1205	1278	1324?	1357?	1377	1469	1510	1670?	1735	1745	1820	1872?	1908	2048	2108	2155	2191
	Recorded #	13				5						2				30			4			4	5
	Amino Acid	2267	2320	2339?	2362	2366	2395	2682	2797	2910	2921	2937	3011	3207	3219?	3312?	3313	3332	3335	3337	3340	3368	3396?
	Recorded #				2	37		2	2	4	3	7	10						2		2		

BMD	Amino Acid	46	82	140	144	158?	163	165	166	168	171?	172	231	277	437	726	882	1131	1136	1318	1469	1745	2155
	Recorded #	4		2									2	2			8					6	
	Amino Acid	2366	2574	2740	2778	2910	2912	2921	3059	3311	3312	3320	3335?	3368	3421								
	Recorded #	13				2	2							4									

DMD/BMD	Amino Acid	89	144	166	199	274	365	409	437	446	518	573	797	882	991	1136	1245	1388	1510	1626	1672	1721	1728
	Recorded #										4			4		2							
	Amino Acid	1745	2010	2035	2039	2108	2155	2366	2395	2607	2654	2910	2912	2921	2937	2951	3032	3232	3319	3320	3335	3340	3383
	Recorded #	2					2	3				3	3		3								
	Amino Acid	3521																					
	Recorded #																						

Notes:

Amino Acid= The amino acid in dystrophin that has a recorded mutation and referenced in the diagram above.

Recorded #= refers to the number of patients that have been submitted for that particular mutation location.

"?"= means diagnosis by source was unsure but significant enough to submit mutation to database.

DMD/BMD =means the patient showed signs of both DMD and BMD so the mutation was submitted as such.

Source: http://www.dmd.nl

amino acid. The amino acid numbers listed in the tables correlate to the amino acid counting from the N-terminus. The 'Recorded #' is the number of patients that have been recorded to possess that specific mutation.

4. Discussion

The dystrophin mutations causing DMD/BMD were very wide spread throughout the protein. The amount of mutations recorded in each structural region was divided by the total amount of amino acids in that domain to determine the percentage mutated in Figure 1. This was done to determine if there was anyone domain region that was key to the proteins function. Based on the results it appears that every region plays a role in the proteins proper function. The highest region of mutation occurrence is 33.19% in the Actin Binding domain (ABD1). Further analysis of ABD1 is being complete currently with dystrophin constructs being analyzed thermodynamically. Looking at only the single substitution mutations determined if there is any one key residue that is important to function as well as the given region in the protein. Looking at data no clear region determined DMD specific or BMD specific. Currently there is not a large enough sample size to do a statistical comparison if a mutation is solely DMD or BMD. DMD reoccurrence for amino acid 1745 was significantly higher than the rest, however this mutation also reoccurred in BMD patient. Further investigation is need on the exact amino acid substitution and property changes to determine if there are significant differences between the mutations at the amino acid residue for DMD and BMD patients. As well as doing informatics in the currently, lab differential scanning calorimetry is being completed on the ABD1 region of dystrophin. This shows the stability differences of ABD1 vs the protein as a whole. How ABD1 responds to changing temperature as well as how stable it is within its environment. The results display a simple mapping of all the current mutations based on domain region and mutation type. From this data no clear correlation between domain region and disease is shown.

5. Conclusion

Currently this research is being continued. Further analysis of the amino acids substitution and regions is being completed as well as the purification of dystrophin mutation constructs to determine thermodynamic significance. This research will be continued throughout this year to determine the key differences between DMD and BMD mutation as well as dystrophin's response to lab rendered mutations.

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